

IN THE SPECIFICATION

Amend the specification as follows.

Page 1, after the title insert the following new paragraph:

"The contents of the attached CD-R compact discs are incorporated herein by reference in their entirety. The attached discs contain identical copies of a file "620-262.TXT" which were created on the discs on May 24, 2004, and are each 615,372 KB."

AMENDMENTS TO THE SPECIFICATION:

Please amend the paragraph beginning at page 6, line 26, as follows:

In one preferred form the method relates to the EHV-1 ORF30-m1 region marker at amino acid position 752 (based on the V592 numbering - see SEQ. ANNEX 1 (SEQ ID NOs:1-104, consecutively) and SEQ. ANNEX 3b (SEQ ID NO:156)) or one which corresponds to this when sequences are aligned as shown herein (see Table 4).

Please amend the paragraph beginning at page 7, line 30, as follows:

The invention thus employs the identity of a codon at nucleotide positions 2254-2256 or 2278-2280 (based on the EHV-1 V592 numbering - see SEQ. ANNEX 1 (SEQ ID NOs:1-104, consecutively) and SEQ. ANNEX 3a (SEQ ID NO:155)) or one which corresponds to this when sequences are aligned.

Please amend the paragraph beginning at page 16, line 34, as follows:

The invention also provides materials which may be used in the methods disclosed herein. These include isolated nucleic acid molecules consisting of the DNA sequence of EHV-1 (strain V592) ORF30 shown in SEQ. ANNEX 3 (SEQ ID NO:155 and SEQ ID NO:156), which sequence comprises a mutation which reduces the virulence of the gene product.

Please amend the paragraph beginning at page 17, line 1, as follows:

Also provided are isolated peptides comprising, or consisting of, or consisting essentially of, a contiguous portion of at least 10, 15, 20, 30, 40, or 50 amino acids of the amino acid sequence of an EHV-1 strain (preferably strain V592) ORF30 shown in SEQ. ANNEX 3 (SEQ ID NO:155 and SEQ ID NO:156), wherein the portion includes position 752 or 760 of SEQ. ANNEX 3 (SEQ ID NO:155 and SEQ ID NO:156).

Please amend the paragraph beginning at page 17, line 30, as follows:

Preferred primers for the amplification of regions of variable sequence for several ORFs are shown in SEQ. ANNEX 2. (SEQ ID NOs:105-154, consecutively) Of these, the primers for amplification of the ORF30-m1 marker region (ORF30f, ORF30r) and of the ORF68 marker region (ORF68f, ORF68r) are particularly preferred.

Please amend the paragraph beginning at page 23, line 31, as follows:

Where the method involves PCR, or other amplification procedure, any suitable PCR primers may be used. Example primers are described herein. For example primers are shown in SEQ. ANNEX 2 (SEQ ID NOs:105-154, consecutively) Preferred primers are any of those listed as ORF30f, , ORF30r,, ORF68f and ORF68r.

Please amend the paragraph beginning at page 24, line 37, as follows:

Preferred sequencing primers include any of those shown in SEQ. ANNEX 2 (SEQ ID NOs:105-154, consecutively), particularly ORF30s, ORF68s1, ORF68s2 and ORF68s3.

Please amend the paragraph beginning at page 27, line 35, as follows:

During analysis of the V592 sequence data a number of positions were noted where there were differences between the sequences determined from individual sequence templates, indicating that the V592 virion DNA population was heterogeneous at these positions. Each of the regions is listed (numbered according to the V592 genomic sequence, SEQ. ANNEX 1a (SEQ ID NO:1)) and details of the variation noted. NA indicates that the position lies outside of a recognised protein coding sequence (open reading frame: ORF).

Please amend the paragraph beginning at page 28, line 7, as follows:

V592 ORFs which have an altered amino acid sequence compared to the corresponding AB4 ORF are listed. The position of each change is numbered according to the V592 ORF amino acid sequences (SEQ. ANNEX 1b (SEQ ID NOs:2-104, consecutively)). For each position, the amino acid sequences for AB4 and V592 are shown. * indicates that the corresponding sequence is absent in the

given strain – i.e. the other strain carries additional amino acids, usually due to variation in copy number of a nucleotide repeat element.

Please amend the paragraph beginning at page 28, line 33, as follows:

Various herpesvirus DNA polymerase sequences were aligned using CLUSTALW (using services at ANGIS, Australia) and the alignment surrounding the EHV-1 ORF30-m1 (D/N₇₅₂) position is shown. Gaps are indicated by dashes (-). Viruses have been grouped as alpha-, beta- and gammaherpesviruses, or unclassified. In each case the abbreviated virus name and sequence accession number are shown (apart from EHV-1 strain V592, reported in this study (SEQ. ANNEX 3b (SEQ ID NO:156))). The first position of each sequence is numbered; where the complete DNA polymerase sequence is not available, numbering of the partial sequence is shown in italics. The position of the D residue conserved in the majority of viruses, corresponding to the ORF30-m1 (aa752) marker position, is shaded.

Please amend the paragraph beginning at page 29, line 25, as follows:

SEQ. ANNEX 1. a) Complete genomic sequence of EHV-1 strain V592 (SEQ ID NO:1); b) Feature table (SEQ ID NOs:2-104, consecutively)

Please amend the paragraph beginning at page 30, line 3, as follows:

SEQ. ANNEX 2. PCR amplification and sequencing primers (SEQ ID NOs:105-154, consecutively)

Please amend the paragraph beginning at page 30, line 9, as follows:

SEQ. ANNEX 3. a) DNA sequence of EHV-1 (strain V592) ORF30 (SEQ ID NO:155); b) Amino acid sequence of EHV-1 (strain V592) ORF30 (SEQ ID NO:156)

Please amend the paragraph beginning at page 30, line 20, as follows:

We determined the V592 genomic sequence (149,430 bp) via shotgun cloning and sequencing of viral DNA prepared from purified virions. Virus supernatant was prepared from equine embryonic lung cells infected at low multiplicity (<0.001 pfu/ml), virions purified by sucrose gradient sedimentation and DNA extracted from purified virions essentially as described by Telford et al [19]. Viral DNA was self ligated, sonicated to generate random fragments and, following end repair, cloned into M13mp19. In addition, a panel of 'semi-random' clones was generated by digestion of viral DNA with frequent cutting (blunt ended) restriction enzymes (AluI, PvuII, BalI), followed by cloning into M13mp19. In the final stages of sequencing, specific regions spanning sequence data 'gaps' were amplified by PCR, blunt ended and cloned into M13mp19. Single stranded M13 templates were prepared and sequenced using proprietary sequencing reagents, and samples analysed on either an ABI 377 or ABI 9600 automated sequencer. Sequence reads were

assembled using the Staden sequence analysis programs PREGAP4 and GAP4 [21]. The complete genomic sequence of EHV-1 strain V592 is shown in SEQ.

ANNEX 1 (SEQ ID NOs:1-104, consecutively). It should be noted that the attached sequence is the consensus for the majority of sequence reads. The V592 stock used to generate DNA for sequencing has not been plaque purified and consequently contains a mixture of DNA populations at certain sites. Sites showing heterogeneity between individual sequencing templates are shown in Table 1. These sites are all regions of variable repeat length apart from one position of single nucleotide heterogeneity.

Please amend the paragraph beginning at page 32, line 15, as follows:

In order to test the above hypotheses, we assembled DNA samples prepared from a panel of field isolates (from the U.K. and North America) recovered from outbreaks of varying disease severity, collected over the course of 30 years. A subset of the ORFs with observed coding changes were selected for preliminary analysis, namely ORFs 8, 11, 14, 15, 30, 33, 34, 37, 39, 40, 52, 67, 68, 73. For each of these ORFs, PCR primers were designed for amplification of regions of sequence variability and for sequencing, as listed in SEQ. ANNEX 2 (SEQ ID NOs:105-154, consecutively). PCR products were purified and sequenced (ABI 9600) and the results assembled using the DNASTAR software package. For each ORF region analysed, positions of variable sequence were noted as listed in Table 5.

Please amend the paragraph beginning at page 35, line 23, as follows:

Notably, this is not the case for one of the variable sequences within ORF30. The complete nucleotide and amino acid sequence of EHV-1 strain V592 ORF30 is shown in SEQ. ANNEX 3 (SEQ ID NO:155 and SEQ ID NO:156). Three nucleotide changes are present compared with the AB4 ORF30 sequence (accession no. AAB02465), namely C₉₂₄-T (non-coding change), G₂₂₅₄-A (amino acid change D₇₅₂-N) and G₂₉₆₈-A (amino acid change E₉₉₀-K).

Please amend the paragraph beginning at page 39, line 38, as follows:

The PCR mix (50µl) consisted of 0.3µM of each primer (Genset), 0.2mM of each NTP (Applied Biosystems), 3X PCRx Enhancer solution (Invitrogen) and 1.25U/µl AmpliTaq DNA polymerase (Applied Biosystems) in 10 mM Tris-HCL (pH 8.3) solution containing 1.5mM MgCL₂ (Applied Biosystems). The PCR reaction was denatured for 4 min at 94°C, then cycled for 32 cycles at 94°C for 30 seconds, 1 min at the annealing temperature of the primers used, and 2 min at 72°C followed by a final step of 10 min at 72°C. After cycling, 10µl of each PCR product was size fractionated on a 0.7% agarose gel containing ethidium bromide. Following product identification, the PCR products were purified using an Amicon Microcon Filter YM100 kit and quantified by size fractionation, on a 2% agarose gel containing ethidium bromide, with DNA Quantification standards (Whatman Bioscience). The purified PCR products were either cloned into M13mp19 (gap filling for

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determination of V592 sequence) or directly sequenced using EHV-1 specific
sequencing primers (SEQ. ANNEX 2 (SEQ ID NOs:105-154, consecutively)).

Replace the text of pages 45 and 46 in their entirety with the following:

Table 2 - ORF coding changes between V592 and AB4

| ORF | Position ¹ | AB4 sequence | V592 sequence |
|-----|---|--|--|
| 2 | 59 | G | D |
| 5 | 114 | G | V |
| 8 | 114 | D | N |
| 11 | 189 | Q | K |
| 13 | 305 460 | S A | L T |
| 14 | 619-621 | * | PSR 9bp duplication resulting in 3 amino acid insertion |
| 15 | 166 | D | N |
| 22 | 430 | S | P |
| 24 | 2567-2568 2586 2829-2836 2904 2913-2927 3099 | (PTLPPAPPLPQSTSKAASGPP (SEQ ID NO:157)) ₂ G * E * T | * 126bp deletion (2 copies 63bp repeat element) resulting in 42 amino acid deletion S AKDQAKDQ (SEQ ID NO:158) 24bp insertion (2 copies 12bp repeat element) resulting in 8 amino acid insertion K PTGAVPENTPLPDDS (SEQ ID NO:159) 45bp insertion resulting in 15 amino acid insertion A |
| 29 | 12 | T | K |
| 30 | 752 990 | D E | N K |
| 31 | 90 | N | S |
| 32 | 42 | S | L |
| 33 | 15 976 | N N | H D |
| 34 | 66 | D | G |
| 36 | 47 | S | R |
| 37 | 265 | A | V |
| 39 | 440 | S | L |
| 40 | 196 | R | H |

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| | | | |
|----|---------|----|---|
| 42 | 1275 | K | R |
| 45 | 427 | E | G |
| 46 | 140 | F | S |
| 50 | 367 | P | S |
| 52 | 386 | A | V |
| 57 | 804 | K | R |
| 64 | 73 | T | A |
| | 648 | T | S |
| 67 | 261 | S | F |
| 68 | 210 | R | H |
| | 247 | D | M – due to single nucleotide (C) deletion resulting in frameshift relative to AB4. All downstream sequence divergent from AB4. Resulting polypeptide 303 amino acids long (cf. 418 for AB4) |
| 71 | 226-227 | SS | TA |
| | 231-299 | * | TAATTTAATTSSATTAATTSS(TTTAA) ₉ TTT (SEQ ID NO:160) additional copies 15bp repeat elements and nucleotide substitutions, resulting in 69 amino acid insertion ² . |
| 73 | 122 | A | V |
| 76 | 128 | F | S |

- 1 Numbered according to the V592 amino acid sequence.
- 2 Variable copy number in V592 – majority sequence shown

Replace the entirety of Table 4 on page 48 with the following new Table 4:

Table 4 - Amino acid sequence alignment for herpesvirus DNA polymerases (SEQ ID NOS:161-197, consecutively)

| Virus | Accession | | |
|--------------|-----------|-----|---|
| ALPHA | | | |
| EHV-1 V592 | | 738 | ALDEVDLAGLQPS-----VNYSTFEVGDQK-LFFVHAHIRESL |
| EHV-1 AB4 | P28858 | 738 | ALDEVDLAGLQPS-----VDYSTFEVGDQK-LFFVHAHIRESL |
| EHV-4 | AAC59546 | 739 | ALNEVDLAGLQPC-----VDYSTFEVGDQK-LFFVHAHIRESL |
| BHV-1 | CAB01595 | 770 | VRREAAPAGLTPG-----ADYATFDVGGRA-LHFVRAHVRESLL |
| BHV-2 | AAD55134 | 739 | ALDAEAVGGLEAG-----RDYMEITVGGDT-VYFVKAHVRESLL |
| FeHV-1 | CAA12264 | 727 | TTERQSLETLRPG-----IDFSEFDVGGHK-LYFVDSHVREEPA |
| VZV | P09252 | 702 | TLNFETVKRLNP-----SDYATFTVGGKR-LFFVRSNVRESLL |
| SimVZV | AAG27201 | 688 | AFDIESVKHLGS-----NDYSVFNVGGQQ-LFFVHAHIRESL |
| PRV | AAA74383 | 593 | ALAR--PAGLRE-----DEFSAFEVNGER-LYFVHAGVRESLL |
| HSV-1 | P09854 | 737 | SLRADAVAHLEAG-----KDYLEIEVGGRR-LFFVKAHVRESLL |
| HSV-2 | P07918 | 742 | SLRPEAVAHLEAD-----RDYLEIEVGGRR-LFFVKAHVRESLL |
| MDV-1 | AAG14223 | 718 | VHDDTNLSNLRPQ-----DDYLEINVQGKL-LRFVKPHIRESLL |
| MDV-2 | BAA78719 | 690 | INDDRKLADLRPR-----DDYMEIDVQGKS-LHFAKPHIRESLL |
| HVT | AAG45768 | 703 | LPAGTIINDLRRG-----DDYIEIDVQGSIL-LRFVKPHIRESLL |
| BETA | | | |
| HCMV | DJBEC1 | 737 | LVPGGEYPVDP-----ADVYSVTLENGVTHRFVRASVRVSVL |
| MCMV | AAA45940 | 644 | LVE--GSPEVP-----EKDVLRVEIGDQC-HRFVRENVHRSLL |
| RCMV | Q85428 | 670 | LVD--GSPPVP-----DEDVLEVVGATRYRFVREHVRSLL |
| PorcCMV | AAF80109 | 587 | ILNDEDVTGID-----EKDILTVMKNTVYRFVRSVRESML |
| RhCMV | AAC05256 | 611 | VAPGGESPPE-----SDVLTVELESGLSYRFVKNVRSNL |
| GPCMV | Q69025 | 666 | LPL--GRDDG-----LSDDDVFLLEFDDGTRYGFVREHVRSIL |
| ElephHV-1 | AAG41999 | 625 | ITDNYVASLR-----EEDITMVTNTGRVHRFVKPHVRSIL |
| HHV-6 | NP_049231 | 592 | VLDERQIAGLS-----ESDILTVKLGD-ETHRFVKPCIRESVL |
| HHV-7 | AAC40752 | 592 | VVDENAVIGLH-----ADDILTVMHVGVP-VTHRFVKKTVRESIL |
| GAMMA | | | |
| EBV | NP_039908 | 604 | ITPGEEHRLA---GLRPGEDYESFRLTGGV-YHFVKKHVHESFL |
| CalliHV3 | AAK38212 | 601 | VTPGEEGKLR---DLRPGEDYESFSLSGGT-FHFVKKHIIKSFL |
| BHV-4 | AAK07928 | 600 | IQDQNLHLH---HLKPD-DYETPHLSTGP-IHFVKQHKTKSLL |
| AlHV-1 | AAC58060 | 622 | IKQQDLPKFT---NLTA-DYETFMISGGP-VHFVKKHKTESLL |
| MHV-68 | AAF19277 | 601 | IPDNKLQMFP---NLTPA-DYETFTLPSGT-VHFVKKHKKCSLL |
| HVS | DJBEM2 | 594 | IPHHALHNYP---HLKSS-DYETFMLSSGP-IHFVKKHIIQASLL |
| AtelHV3 | AAC95533 | 594 | IPHNSLHNYP---YLKSS-DYETFMLSSGP-IHFVKKHIIQTSLL |
| HHV-8 | AAB62593 | 605 | IPGDSLHLHP---HLSPD-DYETFVLSSGP-VHFVKKHKRESLL |
| PorcLTHV-1 | AAF16520 | 592 | IHHEDLHKYP---QLKEE-DYETFLISSGP-VHFVKKHIISESLL |
| EHV-2 | NP_042605 | 602 | IPGDRCLLHP---HLGPG-DYETFELASGP-VHFVKKHKAVSLL |

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|---------|-----------|-----|---|
| GTHV | AAC26681 | 14 | TRRAETLK-----ELKAGEDYEEFKVQGMS-LFYVKPHVRRSLL |
| TortHV | BAB40430 | 14 | TRNPESLK-----DLKAGKYVSFNVQGHT-LYYVLNHVKQSLL |
| CCV | NP_041148 | 664 | DSDKTNRV-----GDYMGYDWSKIDQGF EK-FTLVLRVDRTDPE |
| RanHV-1 | AAD12269 | 690 | DVRRVAQF-----RGWIVFDWRQIEEGFGL-ASLMYTPSKRRFL |

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Replace the entirety of Table 5 on pages 49-54 with the following new Table 5:

Table 5 - Variable Sequence Marker Codes

ORF 8

CODE 331
1 CGTCTCTCGG (SEQ ID NO:198)
2A (SEQ ID NO:199)

ORF 11

CODE 560
1 AGAGTCAGTG (SEQ ID NO:200)
2A.... (SEQ ID NO:201)

ORF14

| | | | | | | | | |
|------|----------------------------|-----------------|----------|-----------|------------|------------|-------------|-----------------|
| CODE | 1841 | 1851 | 1861 | 1871 | 1881 | 1891 | 1901 | |
| 1 | TGCGCCCCAG C-----CGTGCCGGG | CGTCCCGG | CGTCCCGG | TGAGAGTGA | AGACCAAACT | CTGGAACCAT | CGTCCCCCGC | (SEQ ID NO:202) |
| 1A | | | | ...A..... | | | | (SEQ ID NO:203) |
| 1B | | | | ...C..... | | | | (SEQ ID NO:204) |
| 2 | | .CGCCCCAGC..... | | | | | | (SEQ ID NO:205) |
| 2A | | .CGCCCCAGC..... | | ...A..... | | | | (SEQ ID NO:206) |
| 2B | | .CGCCCCAGC..... | | | | |A..... | (SEQ ID NO:207) |
| 3 | | .CGCCCCAGC..... | | | | | | (SEQ ID NO:208) |

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ORF15 490

1 AACCTCGATG (SEQ ID NO:209)
2A... (SEQ ID NO:210)

ORF30-m1

| CODE | 2251 | 2261 | 2271 | 2281 | 2291 | 2301 | |
|------|------------|----------|----------------|-------------|-------------|------------|-----------------|
| 1 | GTCGACTACT | CGACGTTT | CGA GGTGGGTGAC | C AAAAGTTAT | TTTTTGTCCA | CGCCCATATT | (SEQ ID NO:211) |
| 1A |C.. | | | | | | (SEQ ID NO:212) |
| 2 | ...A..... | | | | | | (SEQ ID NO:213) |
| 2A | ...A..... | | | |A..... | | (SEQ ID NO:214) |
| 2B | ...A..... |G. | | | | | (SEQ ID NO:215) |

ORF30-m2

CODE 2961
1 GGCAGCAGAG (SEQ ID NO:216)
2A.. (SEQ ID NO:217)

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ORF33-m1

CODE 41

1 GCAATTGGCG (SEQ ID NO:218)
2 ..C..... (SEQ ID NO:219)

ORF33-m2

CODE 2921

1 TGGAAAATGA (SEQ ID NO:220)
2G.... (SEQ ID NO:221)

ORF34

CODE 151 ^ 191 ^ 301
1 CAACAGACAA ^ CGGACGATCT ^ GCCTGCCGGG (SEQ ID NOS:222, 224 and 226, respectively)
2T.... ^ (SEQ ID NOS:223, 224 and 226, respectively)
2AT.... ^ ..A..... (SEQ ID NOS:223, 224 and 227, respectively)
2BT.... ^T.... (SEQ ID NOS:223, 224 and 228, respectively)
3 ^G... ^ (SEQ ID NOS:222, 225 and 226, respectively)

ORF37

CODE 791 801 811 821 831 841 851
1 GGGGGCGTC CCTTTTTC CCAAAATAA agcgggtgca attaaagacg agtgcacctt tttt-gtggc (SEQ ID NO:229)

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1A (SEQ ID NO:230)
2 ...T..... (SEQ ID NO:231)

ORF39

CODE 1311 ^ 1561
1 AATAGTGTCA ^ CCCGAGCCAG (SEQ ID NOS:232 and 234, respectively)
2 ^C. (SEQ ID NOS:232 and 235, respectively)
3T. ^C. (SEQ ID NOS:233 and 235, respectively)

ORF40

CODE 491 ^ 581
1 TCTACACCCC ^ TTGATCGTAT (SEQ ID NOS:236 and 238, respectively)
2T.. ^A... (SEQ ID NOS:237 and 239, respectively)

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ORF52

CODE 1151

1 GATAGGCCAA (SEQ ID NO:240)
2T... (SEQ ID NOP:241)

ORF67

CODE 751 781

1 GCCAGGCAGC ^ TCTGCAGAAA (SEQ ID NOS:242 and 244, respectively)
1AA. ^ (SEQ ID NOS:243 and 244, respectively)
2 ^ .T..... (SEQ ID NOS:242 and 245, respectively)

ORF68

CODE 331 341 ^ 621 ^ 701 711 721 731 741 751
1 CATCTCAACT CCAGCCTTAT ^ ATTAGTTTCTG ^ GCGGGCCGCT GCCCGGGCGG CGGCCGTCGG AGGGGGGGG-A TCGCGGCCCC
GAGCGGCGC (SEQ ID NOS:246, 249 and 251, respectively)
1A ^ ^ ^G.
..... (SEQ ID NOS:246, 249 and 252, respectively)
2 ^ ^ ^
..... (SEQ ID NOS:246, 249 and 251, respectively)
2A ^ ^C
..... (SEQ ID NOS:246, 249 and 253, respectively)

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3 ^A. ^T.
..... (SEQ ID NOS:246, 250 and 254, respectively)
3A ^A. ^T.
..... (SEQ ID NOS:246, 250 and 255, respectively)
4 ^A. ^
..... (SEQ ID NOS:246, 250 and 251, respectively)
4A ^A. ^
..... (SEQ ID NOS:247, 250 and 251, respectively)
4B ^A. ^
....T..... (SEQ ID NOS:246, 250 and 256, respectively)
5 ^A. ^G..A.....
..... (SEQ ID NOS:246, 250 and 257, respectively)
6T.... ^A. ^
....T..... (SEQ ID NOS:248, 250 and 256, respectively)

ORF73

CODE 360

1 CAATGCCTCT (SEQ ID NO:258)
2T.... (SEQ ID NO:259)